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## THE STORY OF VITAMIN K

Six years ago, in May 1937, an editorial in the *Journal of the American Medical Association* drew attention to the existence of a newly-recognized vitamin. Although sounding a note of caution, the announcement heralded the approach of a new era in which a greater understanding of the mystery of blood-clotting was destined to be developed:

> "A new vitamin-like substance is apparently in process of gradual evolvement. Its qualities are antihemorrhagic, and tentatively it is called vitamin K. It is obtained in relative abundance from alfalfa. Its structure is said to be complex. In vitamin K avitaminosis it is prothrombin that is virtually absent, and the possible relationship between the two is of considerable interest. Great caution is indicated in drawing conclusions from human cases of any type showing hemorrhagic symptoms. In capable hands the exploration of the possibilities of this new vitamin may lead to the further elucidation of the mystery of the clotting of blood."

Since that time much of the prophecy has been fulfilled. Vitamin K has found its place as a new vitamin in human nutrition. It is now recognized as a specific in the treatment of certain diseases characterized by failure of the blood to clot in normal fashion. It is responsible for saving the lives of thousands of new-born babies and has materially decreased the hazards of hemorrhage in modern surgery. The use of vitamin K, in fact, has become routine in surgical practice and in obstetrics.

The events preceding the announcement of the new vitamin and the amazing advances made so soon thereafter constitute a story as dramatic as any of those relating to the original discovery of the vitamins as vital factors in the physiology and nutrition of all living things. The story began, not so long ago, in 1929. Within the space of ten years, the existence of the new vitamin was confirmed, its functions clearly delineated, the vitamin itself isolated in pure crystalline form, and its chemical formula and structure determined. Vitamin K was finally created synthetically in the laboratory. Synthesis of the vitamin, however, did not mark the end of the story. In the years following, numerous other compounds having vitamin K activity were synthesized. Many of these are simpler in structure and possess the much-desired property of water-solubility, which is of invaluable importance in problems of clinical administration. This aspect of vitamin K research is of special interest inasmuch

as hitherto no simple compounds corresponding in chemical structure to the chemically identified vitamins have ever been found which exhibit the same or even greater order of activity as the vitamins themselves.

Vitamin K occurs naturally in two forms: $K_1$ in plants and $K_2$ in certain microorganisms. It is found in relatively small amounts in animal products, the most significant animal sources being hog liver fat, milk, and eggs. Man and other mammals normally do not depend upon food as the sole source of vitamin K inasmuch as a large portion of their needs is supplied through the synthesizing action of bacteria in the intestinal tract. An important exception to this rule occurs in the case of newborn infants who frequently depend upon the administration of preformed vitamin K either to the mother during labor or to the infant itself after birth. The successful use of powdered milk as a natural source of vitamin K for newborn infants has also been reported.

## *The Mechanism of Blood-Clotting*

Before the significance of vitamin K, the "blood-coagulation" vitamin, can be appreciated, it is necessary to know something of other factors involved in blood-clotting and the relation of vitamin K to these factors. There are many factors influencing this reaction about which little is known and whose existence, as in the case of vitamin K, has only recently been suspected. Many theories have been advanced to explain the interaction of these various factors in the production of a blood-clot. In simplified terms, most of these theories agree that:

1. There are, among the many constituents of the blood, two groups of substances directly involved in the clotting mechanism. These are *prothrombin, calcium, fibrinogen* on the one hand and *anti-thrombin* (*heparin*) and related substances on the other hand. The latter group maintains the blood clotting agents in an inactive state, permitting normal circulation of the blood.
2. The clotting process is initiated upon injury by the release of a substance from the injured cells. This substance is *thromboplastin*. It neutralizes heparin thereby leaving prothrombin, calcium and fibrinogen free to perform the clotting process.
3. Prothrombin, in the presence of calcium salts, is converted to thrombin.
4. Thrombin has the property of coagulating the soluble protein, fibrinogen by converting it to the insoluble form known as *fibrin*.
5. Fibrin forms a network of insoluble material which enmeshes the blood corpuscles forming a red, gelatinous, shrunken solidified mass. This is the blood clot.

Expressed schematically:
a) prothrombin + calcium salts → thrombin
b) fibrinogen + thrombin → fibrin (blood clot)

## *The Discovery of Vitamin K*

The existence of an antihemorrhagic food factor was first suspected in 1929 by Henrik Dam (1) then at the University of Copenhagen, now at the University of Rochester. During the course of his studies on fat metabolism, he observed that newly-hatched chicks reared on a special fat-free diet developed a tendency to bleed. The unusual hemorrhagic syndrome developed within two

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or three weeks and was characterized by bleeding from the pinfeathers, hemorrhages into the muscles and subcutaneous tissues, and gizzard erosions. The ability of the blood to clot seemed to be lost almost entirely. From 1929 onward, the scurvy-like disease was observed by many other investigators, but it was not until 1935 that progress was made in ascertaining the cause of the disease. At that time, Dam (2) showed that a cure could not be effected by means of vitamin C or any of the other known vitamins and concluded that the disease was caused by the absence of a hitherto unknown dietary factor for which he suggested the Danish name, "Koagulations Vitamin," or vitamin K. The new factor was found to be especially prevalent in hog liver fat, alfalfa, green vegetables such as spinach and kale, and in certain cereals.

Schønheyder (3) working with Dam, found the bleeding in chicks to be associated with a decrease in the concentration of prothrombin in the blood plasma and showed that upon administration of materials containing vitamin K, bleeding ceased and the concentration of prothrombin rose to normal.

Coincident with the work of the Danish investigators, American scientists were engaged in similar studies on the new antihemorrhagic factor. A nutritional disease in chicks, characterized by hemorrhagic symptoms similar to those described by Dam, was noted by Holst and Halbrook (4) at the University of California who found that fresh cabbage, alfalfa or fish meal, a common ingredient of certain animal feeds, prevented the disease. Later, the interesting observation was made by Almquist and Stokstad (5) of the same laboratory that protein material apparently free from vitamin K, rapidly developed vitamin K potency if moistened and allowed to stand exposed to room temperature for several days. Following up this observation, they demonstrated that vitamin K is produced by bacterial action and that it is generally present in the lower portion of the intestine even when the animal is maintained on a vitamin K-free diet. Absorption from the lower part of the intestine is minimal in the chick, thus explaining the occurrence of the hemorrhagic symptoms on vitamin K-deficient diets. In mammals, however, absorption does occur in the lower intestine and consequently vitamin K deficiency is rare except in cases where absorption is impaired. This was shown two years later in 1937 by Dam and co-workers (6) who extended their vitamin K studies to various species of animals. They succeeded in producing the hemorrhagic disease in chicks, ducklings, goslings, and, in a mild form, in canaries and pigeons. Attempts to produce the disease in dogs, rats and guinea pigs, however, failed.

Evidence for the indispensability of vitamin K in the human diet was as yet lacking, although Dam (6) advanced the hypothesis that

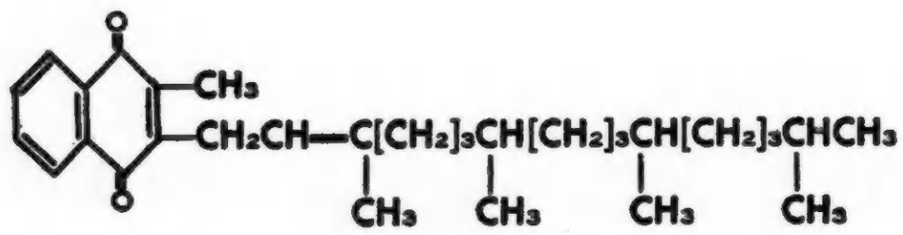
> "Some diseases which are characterized or followed by retarded clotting power might perhaps be supposed, in some way or other, to have a relation to lack of vitamin K due to insufficient synthesis or insufficient absorption from the intestine."

Almost coincident with Dam's statement, the use of vitamin K in cases of bleeding associated with obstructive jaundice or biliary fistula in man was

proposed by Armand Quick (7) in the United States. Quick's proposal was made on theoretical grounds, based (a) on the observation that a greatly reduced prothrombin level of the blood is characteristic of these diseases, and (b) on the fact that vitamin K influences the prothrombin level. Three months after Quick's hypothesis was made, the possible significance of vitamin K in human nutrition was announced in an editorial appearing in the Journal of the American Medical Association (see page 1). Scarcely a year later, the need for caution expressed in the editorial was removed when reports from the University of Iowa (8), the Mayo Clinic (9) and Copenhagen (10) indicated successful use of vitamin K concentrates in the treatment of human cases.

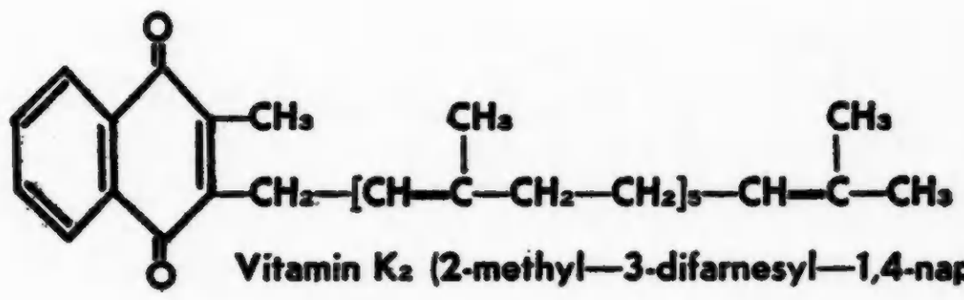
## *The Isolation and Identification of Vitamin K*

From 1938 on, great strides were made in the study of vitamin K. As a results of the combined efforts of scientists both here and abroad, vitamin K was finally isolated, chemically identified and synthesized. The starting point in the chemical studies of vitamin K was alfalfa from which the most potent concentrates were available. Early researches in the purification of such concentrates were led by Dam and co-workers (11, 12) in Europe and Almquist and co-workers in the United States (13) beginning in 1936. Following their initial studies various groups simultaneously pursued the isolation of the pure vitamin. Notable among these brilliant researches was the work of Dam, Karrer et al (14) abroad, of Doisy, McKee, Binkley, Thayer and MacCorquodale (15) at St. Louis, Fieser and associates (16) at Harvard with the assistance of the Merck Institute for Therapeutic Research, Ansbacher and Fernholz (17, 18) at the Squibb Institute, and Almquist et al at California. Almost simultaneously, several of these groups triumphantly announced the isolation of the vitamin which was found to occur naturally in at least two forms: vitamin $K_1$, from alfalfa, and vitamin $K_2$, from putrefied fish meal. Closely following the isolation of the vitamin, the formula and structure were determined and the final step—successful synthesis of the vitamin—was announced by three laboratories in the same issue of the same journal (19, 20, 21) in 1939, a brief three years after the initial chemical studies. Vitamin $K_1$ was identified as a methyl naphthoquinone with a phytyl side chain, having the chemical name 2-methyl-3-phytyl-1,4-naphthoquinone and the structural formula:




**Vitamin $K_1$ (2-methyl—3-phytyl—1,4-naphthoquinone)**

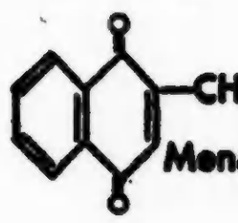
Vitamin $K_2$ has not yet been synthesized, but its structure has been determined. It has been assigned the same formula as vitamin $K_1$ but with a difarnesyl group in place of the phytyl side chain.




Vitamin $K_2$ (2-methyl—3-difarnesyl—1,4-naphthoquinone)

Almquist et al made one of the most extraordinary discoveries in the chemistry of vitamin K namely, that a simple quinone pigment—*phthiocol*—isolated from the tubercle bacillus by Anderson *et al* at Yale University (22) exhibits vitamin K activity. Phthiocol differs from $K_1$ in that the long phytyl side chain is replaced by a simple hydroxyl group.

Phthiocol, although found less potent than vitamin $K_1$, (23) was successfully used in clincal cases at the Mayo Clinic (24). Since then numerous members of the methyl naphthoquinone series, together with their water-soluble salts have been synthesized and successfully used in the treatment of hemorrhagic diseases. The most potent of these is 2-methyl-1,4-naphthoquinone discovered by Ansbacher and Fernholz (25) at a time when the structural formulas of the natural K vitamins were not yet known. These investigators found it to be more highly potent than even the natural K vitamins. The name, *menadione**, is accepted by the Council on Pharmacy and Chemistry of the American Medical Association for this compound.




Menadione (2-methyl—1,4-naphthoquinone)

Perhaps the most significant achievement in the chemistry of vitamin K-active compounds is the development of water-soluble derivatives (26, 27, 28, 29) such as menadiol which greatly facilitate the clinical administration of the vitamin. These water-soluble compounds make the accompanying use of bile salts unnecessary and eliminate the hazard of an oil embolus which might result from intramuscular administration of vitamin K in oil. According to careful studies, these synthetic compounds have been found to be relatively nontoxic and are highly effective from a therapeutic point of view (30).

## *Mechanism of Vitamin K Activity*

Little is known concerning the actual mechanism whereby vitamin K functions in the body. As originally indicated by Schønheyder (3) and by Dam, Schønheyder and Tage-Hansen (31), the failure of the clotting mechanism

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*According to this nomenclature, vitamin $K_1$ becomes *phytyl-menadione*; the water-soluble compound, 2-methyl-1,4-naphthohydroquinone, becomes *menadiol*. All synthetic water-soluble compounds with vitamin K activity are derivatives of *menadiol*.

in the absence of vitamin K is due to a deficiency of prothrombin. Administration of the vitamin rapidly restores the blood prothrombin level from a small percent of its normal value to its usual level with a resultant restoration of blood-clotting power. On the other hand, the direct addition of vitamin K to blood drawn from a deficient chick has no effect upon the clotting time. This would indicate that the role of vitamin K in blood clotting is not a direct one, but that the vitamin is essential for the formation of prothrombin.

The formation of prothrombin is influenced not only by the vitamin K supply, but by the state of the liver which is generally accepted as the site of prothrombin formation. Thus in cases of liver damage, partial removal, or injury by anaesthetics (such as chloroform), a rapid decline in prothrombin results. In such cases, the administration of vitamin K is ineffective.

It has also been found, as will be described later, that interference with the supply of bile will adversely affect prothrombin formation, presumably because the absence of bile from the lower intestinal tract prevents efficient absorption of vitamin K in its fat-soluble forms.

On the basis of these observations, it has been concluded that four basic factors are necessary in the prevention and control of vitamin K deficiency:

1. The presence of bile in normal amounts in the intestinal tract.
2. A liver which is physiologically capable of utilizing the vitamin in the formation of prothrombin.
3. A diet containing the vitamin or materials from which it can be formed by the action of intestinal bacteria.
4. A normal absorptive surface on the intestine.

## *Vitamin K Therapy*

Originally, a vitamin K deficiency could be produced experimentally only in chicks and other fowl. Attempts to induce the hemorrhagic disease in mammals by restricting the vitamin K intake failed. This was correctly interpreted as a result of the ability of mammals to synthesize all their vitamin K needs in the intestinal tract. However, it was soon observed that mammals, including rats, dogs, rabbits and later man gave evidence of vitamin K deficiency under certain pathological conditions. For example, rats (32) and dogs (33) exhibited marked loss of blood clotting power, as well as a low prothrombin level (*hypoprothrombinemia*) when deprived of their bile supply by experimental fistulas. Following this, the observation was made by Greaves and Schmidt (32) that hypoprothrombinemia in experimental animals could be cured partially by the administration of vitamin K and entirely by administration of vitamin K *plus bile salts*. It has also been found that the return of bile to the intestinal tract has a favorable effect on bleeding of patients not having a normal bile supply. This provides evidence for the fact the bile or bile salts are essential to the efficient intestinal absorption of vitamin K.

**Obstructive Jaundice:** Following the realization of the role of bile in the utilization of vitamin K and the original observations by Quick, Stanley-Brown and Bancroft (34) of low prothrombin levels in cases of obstructive jaundice*, other investigators proceeded successfully to overcome the bleeding defect associated with jaundice or biliary fistula by administration of crude vitamin K with bile or bile salts (8, 9, 10). The recent development of water-soluble compounds with high vitamin K activity which can be absorbed directly from the intestinal tract dispenses with the need for bile salts which are highly distasteful and often poorly tolerated by jaundiced patients.

**Other Hemorrhagic Diseases:** It was soon found that vitamin K therapy proved effective in the treatment of hemorrhagic diseases resulting from other causes. These include disorders involving abnormalities of the intestinal surface, as well as conditions resulting from extended sulfonamide therapy. All of these disorders are characterized by hypoprothrombinemia. In certain hemorrhagic diseases, however, vitamin K therapy is of no value inasmuch as the failure of the blood to clot is due to factors other than a decrease in prothrombin. In hemophilia, for example, there is no hypoprothrombinemia and consequently a cure cannot be effected by vitamin K. The defect in hemophilia appears to be a lack of tryptase, a substance essential to the formation of a thromboplastic agent influencing the conversion of prothrombin to thrombin (35).

## *Hemorrhagic Disease of the New-Born*

The period from the second to the fifth day following birth is one of the most hazardous in the life of the infant. During this time all infants are in constant danger of hemorrhage which may be initiated by any one of a number of causes. Most of these hemorrhages begin with a slow internal oozing which cannot be stopped and which eventually causes death. For many years the cause of the hemorrhagic disease of the newborn was sought and assigned to various factors, but none of these proved to be significant.

Cases of low clotting power of the blood of infants were observed as early as 1912 by Whipple (36). Brinkhous *et al* (37) reported, in 1927, that this reduced power is due to a relatively low prothrombin content of infant blood. Waddell, *et al* (38) were the first to report that this condition is common in the newborn and that it can be prevented by administration of vitamin K. In 1939 the interesting observation was made by Quick and Grossman (39, 40) that although prothrombin levels were usually normal at birth, the concentration soon dropped, so that after 24 hours the prothrombin reached dangerously low levels. They then found that the prothrombin level gradually increased until the third day, after which it rose to normal. These findings—the result of studies on 31 normal infants ranging in age from 6 hours to 60 days—coin-

*N.B. In obstructive jaundice, as well as in cases of biliary fistulas, there is an interference with the normal flow of bile from the bile duct to the intestinal tract.

cided remarkably with the delay in clotting observed during the second to fifth day of life. From these observations Quick and Grossman concluded that all normal infants exhibit a definite phase of "physiologic hypoprothrombinemia," between the second and fifth days of life.

Further study led to the conclusion that the lack of prothrombin or prothrombin reserve is due to a deficiency of vitamin K. This has been explained by the fact that at birth the intestinal tract of the infant is sterile and so cannot synthesize the necessary vitamin by bacterial action; further the liver of the newborn infant is unable to secrete sufficient bile and consequently the ability to absorb fats carrying fat-soluble vitamins is extremely limited.

These difficulties can be overcome by administration of vitamin K to the infant at birth or even to mothers prior to and during labor. More satisfactory results are obtained when the vitamin is administered to the mothers so that this is now the generally accepted procedure. Routine adoption of this practice has miraculously reduced infant mortality resulting from all types of hemorrhage due to hypoprothrombinemia.

The usual dosage of vitamin K in the treatment of newborn infants is one milligram. According to Smith and Warner (41), this exceeds the minimum requirements by one thousandfold. Milk, which is not taken until a few days after birth, contains enough preformed vitamin K to meet this very low requirement. Dried milk (5 to 10 gr.) in water fed to infants on the third or forth day gives a good prothrombin response.

**Methods of Determining Vitamin K Deficiency:** Clinical methods of establishing the presence of a vitamin K deficiency are based on the demonstration of a reduction in the concentration of prothrombin. It was shown by Dam and Glavind that the plasma of K-avitaminous chicks, the plasma of jaundice patients and the plasma of chicks from which prothrombin has been partially removed, all have a prothrombin deficiency of about the same magnitude and all require the addition of approximately equal amounts of normal plasma to form a clot in a normal period of time. On this basis it was assumed that a defect in blood coagulation caused by a diminution in the concentration of prothrombin is indicative of a deficiency in the absorption and utilization of vitamin K. Quick, who first called attention to hypoprothrombinemia in jaundice, established this point by the method of adding thromboplastin to recalcified plasma, *leaving prothrombin as the only variable* and then recording the coagulation time of the mixture. This became a standard method for the determination of "prothrombin time" and the body stores of vitamin K. Similar methods and modifications have been developed by other workers, one of the simplest being the so-called "bedside test" or single-stage method of Smith, Warner and associates (43). A more complete method is the two-stage test of Smith *et al* (44) which takes into account all the factors involved in the blood-clotting mechanism as expressed by the diagram on page 2.

## *The Occurrence of Vitamin K in Foods*

The term, vitamin K, is used to designate the two known natural forms, $K_1$ and $K_2$, as well as the numerous synthetic compounds exhibiting vitamin K activity. Strictly speaking, the term applies only to the natural forms, the term "vitamers" (45) having been suggested for the synthetic compounds. The natural vitamins occur chiefly in plants and in microorganisms, and to some extent in animal materials. It has been established that the green leaves of plants contain vitamin $K_1$ and that most bacteria contain essentially the vitamin $K_2$ form. Molds, yeasts and fungi are practically devoid of vitamin K.

Important sources of vitamin $K_1$ are the green leafy portions of alfalfa, spinach, cabbage, kale, cauliflower, and the like. The vitamin is also present in tomatoes, seaweed, and certain cereals. Fruits and vegetables are poor sources. Animal or plant material which has been allowed to putrefy develops vitamin K activity as a result of bacterial action.

The richest animal source of vitamin K is hog liver, most animal materials containing very little. Milk and eggs, both designed for the nutrition of the young, contain small amounts.

## *Animal and Human Requirements:*

The K vitamins are required by all experimental animals and by man. The actual amounts required by the different species are not known. It has been estimated that one to two milligrams of the pure vitamin or a synthetic product of like potency is probably the human daily requirement.

There are two sources of vitamin K for human and animal needs, (1) the naturally occurring vitamin in foods and (2) the vitamin produced by bacterial action in the intestine. Some animals like the chick are unable to utilize the vitamin from intestinal bacterial action and so become deficient on a diet containing little or no vitamin K. On the other hand, mammals, including man, utilize vitamin K formed by bacteria in the intestine; consequently they are not easily rendered avitaminotic by a vitamin K-free diet. Vitamin K dietary deficiency, therefore, is usually not a problem in man.

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The literature on vitamin K is too voluminous to cover in this brief review. For those who are interested, a number of reviews containing literature citations are included. (41, 42, 46—59).

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# *NEWS DIGEST*

**LACTALBUMIN,** one of the proteins of milk, has recently been shown in animal studies to be a superior protein for stimulating the regeneration of red blood cells and hemoglobin following experimentally induced anemia. Rats fed a synthetic diet containing 18% of the total calories as lactalbumin showed excellent growth and maintained normal hemoglobin and erythrocyte values, whereas those fed at a 2.8% protein level grew only slightly and developed a chronic anemia. Animals given 18% protein as a special mixture of milk and whole blood solids also showed good growth and normal blood values. When this mixture was fed at the 2.8% level, however, gradual loss of weight occurred and a severe anemia developed, followed by death in many animals. The feeding of 18% protein as beef blood solids alone resulted in very poor growth and the maintenance of low blood values; when fed at the low 2.8% level, little increase in body weight was found and moderately severe anemia developed. —401

**B COMPLEX** vitamins are now obtainable in large quantities from a new source—*Azotobacter,* a genus of nitrogen-fixing bacteria. It has been found that Azotobacter cells grown in a nitrogen-free sucrose-mineral salts medium have a vitamin B complex content equal to or greater than that of brewer's yeast. When grown in a molasses medium, the Azotobacter produces still greater amounts of biotin, thiamin, and pantothenic acid, but less of nicotinic acid and riboflavin. Recently, a method has been perfected for large scale production of Azotobacter. —402

**FROZEN EGGS** in segmented bar form offer an improved method for home use of this product. The frozen shelled eggs are molded in the form of a bar with segments joined thinly so that amounts equivalent to one or more accurately measured eggs may be broken off and used as required. This overcomes the difficulty of accurately measuring a given number of eggs formerly encountered in the use of bulk frozen eggs. With the new modification portions equalling one or more eggs can be broken off as needed and the unused portion put back in the refrigerator without spoiling. —403

**MILK CASEIN** is one of the few phosphoproteins which equals inorganic phosphorics salts in availability for producing satisfactory bone calcification in animals suffering from experimental rickets. The phosphorus of ether-alcohol extract pork liver and the phosphorus of egg yolk phospho lipid were found to be almost equivalent in availability to casein phosphorus and inorganic phosphorus. The phosphorus of ether-alcohol extract of pork loin and of ovovitellin from egg yolk, on the other hand, appear to be definitely less available for calcification under the same experimental conditions. —404

**THE AMINO ACIDS,** *threonine, leucine, isoleucine,* and *phenylalanine* have now been added to the list of amino acids essential in human nutrition as indicated by additional nitrogen balance studies on human beings. Several months ago, following preliminary studies of this type, the initial announcement was made that *methionine* and *valine,* two of the ten amino acids indispensable in animal nutrition, are indispensable to man as well. (Cf. *Borden's Review of Nutrition Research,* January, 1943). Of the amino acids studied, therefore, this brings the total number of amino acids essential to man to six. It is interesting to note that *histidine* which is essential for animals, has been found not to be required in the diet by man. The significance of the three remaining amino acids is now being studied. —405

**FROZEN MILK** in concentrated form is now being developed for possible use as a commercial product. The process introduces a new form of preserved milk which can be kept indefinitely in the freezing compartment of the refrigerator. The product is reconstituted by adding hot water and then cooling to desired temperature. One pint of frozen concentrated milk is equivalent to about three pints of liquid milk. —406

**ONION POWDER** is frequently used to impart an excellent flavor to foods without the necessity of incorporating more onion than is desired. Recent investigations indicate that onion tops when dried and pulverized, serve as a good substitute for onion powder. Besides having the advantages of excellent flavor and ease of preparation, pulverized onion tops also contribute nutritive value which may be superior to that contributed by the bulbs of the onions. The tops contain significantly higher percentages of total nitrogen, of total ash and especially of calcium. —407

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**CORRECTION: The values for the riboflavin content of cheddar cheese and other foods as listed in item No. 303 of the March issue were given in terms of "micrograms per gram", due to a typographical error. These should have been expressed in terms of *"micrograms per 100 grams".***